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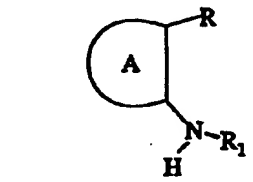
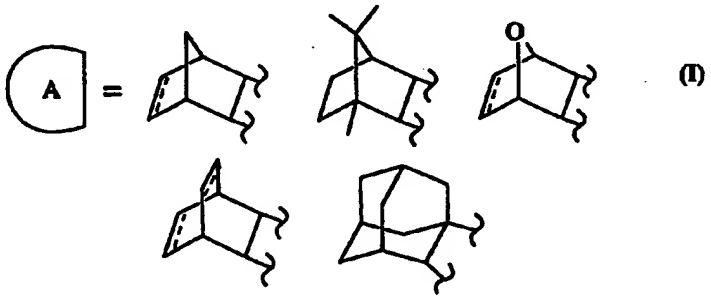
(54) Title: CONFORMATIONALLY RIGID BICYCLIC AND ADAMANTANE DERIVATIVES USEFUL AS α_2-ADRENERGIC BLOCKING AGENTS





(I)

(57) Abstract

A compound of formula (I), in which: ring A is one of the five alternative multi-cyclic rings as shown wherein a dotted line adjacent to a bond indicates that a single bond or a double bond may be present at that position; R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline; R is hydrogen, lower straight or branched chain alkyl of 1 to 6 carbon atoms, or lower straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl; or a stereoisomer, or a pharmaceutically acceptable salt thereof. These compounds have α_2 receptor blocking activity and hence find use in the treatment or palliation of mental depression, elevated intraocular pressure, non insulin-dependent diabetes, male impotence and obesity. Further, these compounds and their primary amine precursors, represented in formula (I) when R_1 is hydrogen are useful as neuroprotective agents in the mammalian nerve cells of the eye and brain.

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Conformationally Rigid Bicyclic and Adamantane Derivatives Useful as α_2-Adrenergic Blocking Agents

Field of the Invention

The present invention relates to aliphatic bridged-cyclic compounds with 2-amino-imidazoline, 2-amino-oxazoline or 2-amino-thiazoline substituents. More particularly, the invention relates to such compounds which are selective in blocking the α_2 adrenoreceptor. These compounds find use in the treatment of conditions which are responsive to regulation of α_2-receptor responses, such activities include, for example, treatment of depression, palliation of non insulin-dependent diabetes, alleviation of male impotence, lowering of intraocular pressure (which is useful in treating e.g. glaucoma) and stimulation of weight loss. Further, it has been discovered that the compounds of the present invention and the primary amine precursors from which these compounds are made have neuro-protective activity.

Background Of The Invention

Adrenergic agents, and particularly agents affective on α_2 adrenergic receptors are known in the art. For example, United States patent 5,091,528 describes 6- or 7-(2-imino-2-imidazoline)-1,2-benzoxazine as α adrenergic agents. Published European patent application 0 251 4543 describes certain cyclohexyl substituted amino-dihydro-oxazoles, -thiazoles and -imidazoles as α_2 agents. United States Patent 3,598,833 describes 2-cycloalkylamino oxazolines having local anesthetic, sedative, vasoconstrictor, mucous membrane de-swelling, blood pressure depressant and gastric fluid secretory inhibition effects. Further United States and foreign patents and scientific publications which pertain to substituted amino-oxazoline, imidazolines and thiazolines are as follows:

United States Patent 4,587,257 [2-trisubstituted phenylimino) imidazoline compounds capable of controlling ocular bleeding];

United States Patent 3,636,219 [2-(substituted-phenylamino)-thiazolines and imidazolines having anticholinergic activity];

United States Patent 3,453,284 [2-substituted anilino)-2-oxazolines;

United States Patent 3,432,600 [partially reduced 2-(naphthylamino) oxazolines and 2-(indanylamino) oxazolines;

United States Patent 3,679,798 [compositions comprising arylaminooxazolines and an anticholinergic agent];

United States Patent 3,624,092 [amino-oxazolines useful as central nervous system depressants];

United States Patent 2,876,232 [2-(9-fluorenylamino)-oxazolines), and German Patent nos. 1,191,381 and 1,195,323 and European Patent Application no 87304019.0;

United States Patent 4,515,800 [2-(trisubstituted phenylimino) imidazoline compounds, also known as 2-(trisubstituted-anilino)-1,3-diazacyclopentene-(2) compounds, for treatment of glaucoma];

United States Patent 5,066,664 [2-(hydroxy-2-alkylphenylamino)-oxazolines and thiazolines as anti glaucoma and vasoconstrictive agents].

Chapleo et al. [Journal of Medicinal Chemistry **1989**, 32, 1627-30] describe heteroaromatic analogs of clonidine as partial agonists of α_2 adrenoceptors.

Poos, et al. [Journal of Organic Chemistry, **1961**, 26, 4898-904.] reported the syntheses of isomeric forms of 2-amino-3-phenylnorbornanes, and that the endo-phenyl-exo-amino compounds demonstrated a biphasic effect on blood pressure. United States Patent 3,514,486 to Hartzler discloses making 3-isopropyl-2-norbornanamine and reports that they have useful antihypertensive activity.

Additionally, commonly assigned co-pending applications 08/186,406 and 08/185,653 disclose alpha-substituted derivatives of aromatic 2-amino-imidazoles and methods of using the same as α_{2A} selective agonists.

Memantine (1,3-dimethyl-5-adamantamine) and adamantamine have been shown to have neuroprotective properties by Kornhuber, et al. [J. Neural Transm., **1994**, Suppl. 43 (Neuroprot. in Neurodegeneration), pp. 91-104].

United States Patent 5,334,618 to Lipton discloses a method for reducing non-ischemic NMDA receptor-mediated neuronal damage in a mammal by using memantine and related adamantamines.

A study by Maiese, et al. [J. Cereb. Blood Flow Metab., **1992**, 12 (1), pp. 53-63] suggested that binding of rilmenidine (a 2-aminooxazole derivative) and idazoxan (an imidazoline compound) have neuroprotective activity as a result of binding to imidazol(in)e receptors. It was found that another non-imidazoline receptor binding α_2 antagonist (SKF 86466) lacked such neuroprotective activity.

Reis, et al. [Am. J. Cardiol., **1994**, 74 (13), pp. 25A-30A] have reported evidence they interpret to show that interactions with central imidazoline receptors are neuroprotective. They have found that imidazoline receptor binding agents which also have α-adrenergic activity have neuroprotective properties whereas an α_2 antagonist which lacks I_1 receptor binding affinity is not neuroprotective.

The background of the division of adrenoceptors into differing categories can be briefly described as follows. Historically, adrenoceptors were first divided into α and β subtypes by Ahlquist in 1948. This division was based on pharmacological characteristics. Later, β-adrenoceptors were subdivided into β_1 and β_2 subtypes, again based on a pharmacological definition by comparison of the relative potencies f 12 agonists. The α-adrenoceptors were also subdivided into α_1 and α_2 subtypes, initially based n a presumed localization of

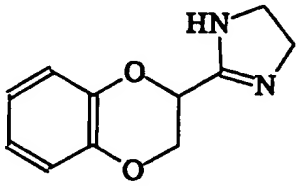
α_1 receptors p stsynaptically and α_2 presynaptically. Now, however, this physiologic division is no longer used and it is generally accepted that the most us ful way to subdivid the α-adrenoceptors is based on pharmacology, using affinities for the α-antagonists yohimbine and prazosin. At α_1 receptors, prazosin is more potent than yohimbine, whereas at α_2 receptors, yohimbine is more potent than prazosin. More recently the α_1 and α_2 receptors have been further subdivided into subtypes such as α_{1A}, α_{1B}, α_{1C}, α_{2A}, α_{2B} and α_{2C}.

The investigation of imidazoline actions independent of adrenoceptors started in the mid-1980's with three key findings. Bousquet (Strasbourg, FR) noted that various imidazolines irrespective of their affinity and efficacy at known α-adrenoceptor subtypes, lowered blood pressure upon injection into a brainstem cardiovascular area, the rostral ventrolateral medulla, whereas catecholamines were inactive. Atlas (Jerusalem, IL) isolated an extract from brain that inhibited [^{3}H]clonidine binding and was hence named 'clonidine displacing substance' (CDS). The laboratories of Reis (New York, US) and Parini (Paris, FR) described [^{3}H]clonidine and [^{3}H]idazoxan binding sites that specifically recognized imidazoline-like drugs but did not bind catecholamines. These binding sites were termed imidazoline receptors and two major subtypes were tentatively designated I_1 and I_2. The 'I' designation is intended to encompass not only imidazolines, but imidazoles and imidazolidines and related structures such as guanidines and oxazolines, all of which are potential ligands at the imidazoline receptor sites. For a short review of imidazoline receptors, see Michel M.C. and Ernsberger P. in Trends Pharmacol. Sci. (ENGLAND), **1992**, 13 (10) pp. 369-70.

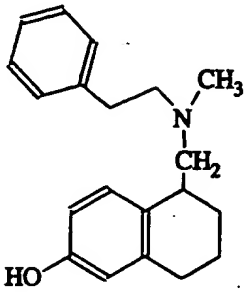
The term agonist refers to a class of compounds which bind with some affinity to and activate a particular type of receptor. Activation refers to what could be considered analogous to flipping on a switch, i.e. the receptor is induced to initiate some kind of action like a physiologic response or a chain of biochemical events. The term antagonist (or receptor blocker) refers to a class of compounds which bind to a receptor with some affinity, but are unable to activate the receptor to provide an effect. The antagonist can be compared to a key

which is able to slide into a lock, but is unable to turn in the lock to open it.

Some examples of alpha$_2$ (α_2) adrenergic receptor blocking compounds known in the art are:



Idazoxan



A-75169

Idazoxan is classified as a selective α_2 antagonist, and has been studied in combination with tyrosine as an antidepressant and in combination with D_2 dopamine receptor antagonists as an antipsychotic agent. 1,2,3,4-tetrahydro-6-hydroxy-1-((N-methyl-amino)-methyl-N-phenylethyl)naphthalene hydrochloride (A-75169) lowers intraocular pressure in mammals.

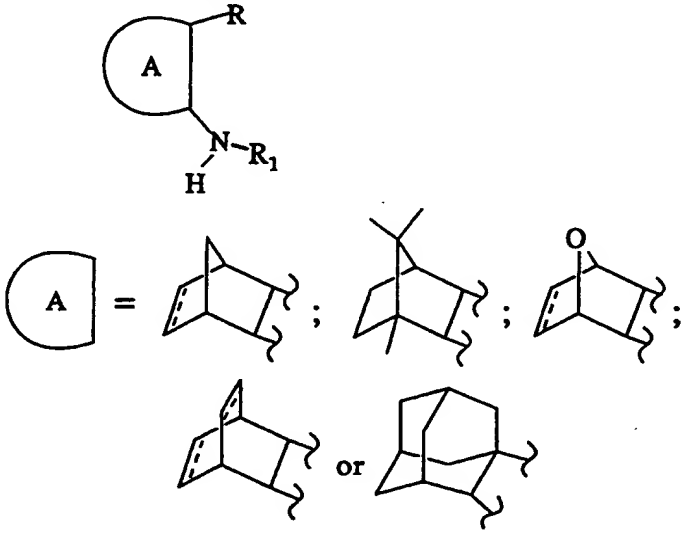
The receptor affinity of candidate compounds can be determined by radioligand binding competition studies. Radioligand binding competition studies assess the affinity of a compound by measuring its ability to displace a radioligand of known affinity.

As described above, an agonist is defined as a compound that binds to and activates a receptor response. An antagonist binds to, but does not activate a response by, the receptor. The measure of activation caused by a bound molecule is said to be its efficacy. Functional experiments are designed to determine whether, after binding, a test compound elicits a biochemical effect, or rather binds without causing the receptor to respond. An antagonist, if of sufficient binding affinity, can be used to block the binding of endogenous molecules in the body which activate a receptor, and thereby prevent its activation. Antagonists can find therapeutic use by blocking the

binding of an oversupply of an endogenous receptor activator or the over expression of a receptor effect. Owing to the intricacy of the interactions between a given binding molecule and the conformation and function of the receptor itself, partial agonists and partial antagonists are also known in receptor pharmacology.

Summary of the Invention

The present invention concerns novel compounds of the formula I,



I

in which: ring A is any of the five alternative multi-cyclic rings shown, R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline and R is hydrogen, straight or branched chain alkyl of 1 to 6 carbon atoms, or straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl. In the drawing of chemical structures as shown above, the intersection of two or more lines indicates a carbon atom, a single line indicates a single bond, and a double line a double bond, and a dotted line adjacent a single line indicates either a single or double bond. The chemical nomenclature for the rings shown above from left to right in descending order is norbornane (or bicyclo[2.2.1]heptane); bornane (or 1,7,7-trimethyl-bicyclo[2.2.1]heptane); 7-oxa-bicyclo[2.2.1]heptane; bicyclo[2.2.2]octane and adamantane (or tricyclo[3.3.1.13,7]decane).

The wavy lines across a bond indicate that the bond attaches to either the R or 2-amino-heterocyclic moieties. Any stereoisomers and diastereomers which are available by bonding th substituents R and the 2-amino-hetero-azole moieties to the available valences of the above-indicated carbons on the rings are contemplated by the invention, as well as the pharmaceutically acceptable salts.

Another aspect of the invention concerns the method of use of these compounds in blocking or antagonizing α_2 receptor function.

A further aspect of the invention concerns methods of using the compounds of formula I and the primary amine precursors which are represented by Figure I when R_1 is hydrogen in protection of nerve cells in the mammalian brain and eye from noxious effects.

Other aspects of the invention relate to pharmaceutical compositions containing the compounds of the invention in admixture with one or more pharmaceutically acceptable, non-toxic carriers, and to methods pertaining to their use.

Detailed Description of the Invention

Definitions

As used herein:

The terms "ester" and "amide" refer to and cover any compound falling within the definition of those terms as classically used in organic chemistry.

The term "alkyl" refers to and includes normal and branched chain alkyl groups as well as cycloalkyl groups. The term "lower alkyl", unless specifically stated otherwise, includes normal alkyl of 1 to 6 carbons, branched-chain alkyl of 3 to 6 carbons and cyclo-groups having 3 to 6 carbon atoms. Similarly, the terms "alkenyl" and "alkynyl" include n rmal and branched chain as w ll as cyclo-alkenyl and alkynyl groups, respectively, having 2 to 6 carbons when

the chains ar normal, and 3 to 6 carbons when the chains are branched or cyclic.

The terms endo and exo are used in describing a substituent in spatial relation to its connection to a bridged ring and refer to the position of the substituent as either "inside" or "outside" the ring. For the bicycloheptane compounds, endo refers to a substituent attached to the ring by a bond that points down and below the general plane of the six membered ring, and exo refers to a substituent attached to the ring by a bond that points out from and above the general plane of the six membered ring.

The terms cis and trans are also used in describing the relative stereochemistry of the substituents of the present invention. Since the carbon atoms at positions 2 and 3 in the norbornane and bicyclo[2.2.2]octane rings are rigidly fixed by the bicyclic ring structure there is no bond rotation or alternative conformation of the ring system. Thus, the bond between carbon atoms 2 and 3 can be likened to a double bond in that respect, and so relative stereochemistry can be described with cis indicating that the substituents are located on the same side of the bond, and trans indicating that the substituents are located in positions opposite one another across the bond.

Neuroprotection and neuroprotective activity refer to the property of sparing nerve cells in the mammalian eye or brain from injury or death following a noxious action or event. Such noxious actions or events can be ischemia, glutamate-induced excitotoxicity, physical trauma or elevated intraocular pressure. Elevated intraocular pressure with subsequent optic nerve damage occurs very commonly in glaucoma.

The mechanism by which these compounds manifests the neuroprotective effect is not yet certain. In the case of memantine and its analogs, NMDA receptor blocking activity is thought to protect cells from glutamate-induced excitotoxicity. Certain comp unds with imidazoline or oxazoline groups which bind to imidazoline receptors

are theorized to have either a direct of remotely induced neuroprotective effect as a result of this binding.

Pharmaceutically acceptable salts of the compounds of formula I are also within the scope of the present invention. Pharmaceutically acceptable acid addition salts of the compounds of the invention are those formed from acids which provide pharmaceutically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, sulfate, bisulfate, phosphate or acid phosphate, acetate, maleate, fumarate, oxalate, lactate, tartrate, citrate, gluconate, saccharate, or p-toluenesulfonate salts. A pharmaceutically acceptable salt may be any salt which retains the activity of the parent compound and does not impart any deleterious or untoward effect on the subject to which it is administered and by the context in which it is administered.

In a method of using the compounds of formula I in neuroprotection, pharmaceutically effective amounts of a protective agent can be administered alone to treat nerve injury or to prevent nerve cell death. The most effective mode of administration and dosage regimen of protective agent will depend on the type of disease to be treated, the severity and course of that disease, previous therapy, the patient's health status, and response to the drug and the judgment of the treating physician. Generally, the neuroprotective agent should be administered in a dose to achieve a serum or intravitreal concentration of 0.01 nM to 50 nM. Preferably the neuroprotective agent is administered prior to injury to the nerve, but can be administered injury has occurred with lessened effect.

Conventional modes of administration and standard dosage regimens of known neuroprotective agents, e.g. MK-801, can be used. Optimal dosages for co-administration of a drug, e.g. an IOP-lowering drug, with a neuroprotective agent can be determined using methods known in the art. Dosages of neuroprotective agents may be adjusted to the individual patient based on the dosage of the drug with which the agent is co-administered and the response of the patient to th treatment regimen. The protective agent may be administered to the patient at ne tim r over a series of treatments.

An agent that cannot pass the blood/brain barrier, e.g. MK-801, may be administered locally, e.g. intravitreally by intrabulbar injection, or intrathecally. Agents which are capable of crossing the blood/brain barrier, e.g. a highly lipophilic molecule, can be administered systemically, e.g., orally, or intravenously, or by injection.

The composition used in these therapies may also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solution or suspension, liposomes, suppositories, injectable and infusible solutions. The compositions also preferably include conventional pharmaceutically acceptable carriers which are known those of skill in the art.

Organic amine salts may be made with amines, particularly ammonium salts such as mono-, di- and trialkyl amines or ethanol amines. Salts may also be formed with caffeine, tromethamine, and similar molecules. Where there is a nitrogen sufficiently basic as to be capable of forming acid addition salts such may be formed with any inorganic or organic acids or alkylating agent such as methyl iodide. Any of a number of simple organic acids such as mono-, di-, or tri-acid may also be used. A pharmaceutically acceptable salt may be prepared for any compound of the invention having a functionality capable of forming such a salt, e.g., an acid salt of an amine functionality.

Utility and dosage forms

The compounds of formula I wherein R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline and pharmaceutically acceptable acid addition salts thereof have been found to possess valuable pharmacologic properties in the central nervous system and, in particular, have been shown to block (antagonize) α_2 receptors in standard laboratory tests. Accordingly, these compounds and pharmaceutically acceptable compositions containing them are useful in reduction or maintenance f the intraocular pressure in at least n eye f a mammal and in regulation of other physiologic phenomena r lated to α_2 receptors. Such physiologic activities includ for example:

alleviation, prev ntion or inhibition of depression in mammals; reduction in the severity of diabetes; alleviati n of male impotence; and stimulation of weight loss.

In applying the compounds of the invention to treatment of diseases or disorders of the eye which are associated with an abnormally high intraocular pressure, administration may be achieved by any pharmaceutically acceptable mode of administration which provides adequate local concentrations to provide the desired response. These include direct administration to the eye via drops and controlled release inserts or implants, as well as systemic administration as described below.

Drops and solutions applied directly to the eye are typically sterilized aqueous solutions containing 0.001% to 10%, most preferably 0.005% to 1% of the active ingredient, along with suitable buffer, stabilizer, and preservative. The total concentration of solutes should be such that, if possible, the resultant solution is isotonic with the lachrymal fluid and has a pH in the range of 6-8. Typical sterilizing agents are thimerosal, chlorobutanol, phenyl mercuric nitrate and benzalkonium chloride. Typical buffers are, for example, citrate, phosphate, borate or tromethamine; suitable stabilizers include glycerin and polysorbate 80. The aqueous solutions are formulated by simply dissolving the solutes in a suitable quantity of water, adjusting the pH with suitable acid or base to a pH of about 6.8 to 8, making a final volume adjustment with additional water and sterilizing the resultant solution.

The dosage level of the resulting composition will, of course, depend on the concentration of the drops, the condition of the subject and the individual magnitude of response to treatment. However, a typical ocular composition could be administered at the rate of about 2 to 10 drops per day per eye of a 0.1% solution of active ingredient.

The compounds of the present invention , when administered for conditions which are regulated by the central nervous system (CNS), can be by any of the accepted modes of administrati n for agents which relieve depression or affect the CNS including oral,

parenteral, rectal, and otherwise systemic routes of administration. Any pharmac utically acceptable mode of administration can be used, including solid, semi-solid, or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, liquids suspensions, or the like, preferably in unit dosage form suitable to single administration of precise dosages, or in sustained or controlled release forms for the prolonged administration of the compound at a predetermined rate. The compositions will typically include a conventional pharmaceutical carrier or excipient and an active compound of formula I or the pharmaceutically acceptable salts thereof and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc.

The amount of active compound administered will course be dependent of the subject being treated, the severity of the affliction, the manner of administration and the judgment of the prescribing physician. However, an effective dosage is in the range of 0.01 - 1 mg/kg/day, preferably 0.1 - 0.5 mg/kg/day. For an average human of about 70 kg, this would amount to 0.7 - 70 mg/day.

For solid compositions, conventional non-toxic carriers include, for example mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, for example, propylene glycol as a carrier. Liquid pharmaceutically administerable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as , for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non toxic auxiliary pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent. to those skilled in this art; f r example, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 15th Edition, 1975.

The composition or formulation to b administered will, in any event, contain a quantity of the active compound in an am unt effective to alleviate the symptoms of the subject being treated.

Dosage forms or composition containing active ingredient of formula I or its salts in the range of 0.25 to 95% with the balance made up from non-toxic carrier may be prepared.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, and may contain 1% - 95% active ingredient, preferably 5% - 50%.

Parenteral administration is generally characterized by injection, whether subcutaneously, intramuscularly, or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspension, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients include, for example, water, saline, aqueous dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions may also contain minor amounts of non-toxic substances such as wetting or emulsifying agents, auxiliary pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

The percentage of active compound contained in such parenteral compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.1% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably the composition will comprise 0.2-2% of the active agent in solution.

Preferred Embodiments

Among the family of compounds of the present invention, a preferred group includes compounds of formula I wherein X is oxygen, i.e. compounds where the oxazoline ring constitutes the heterocycle.

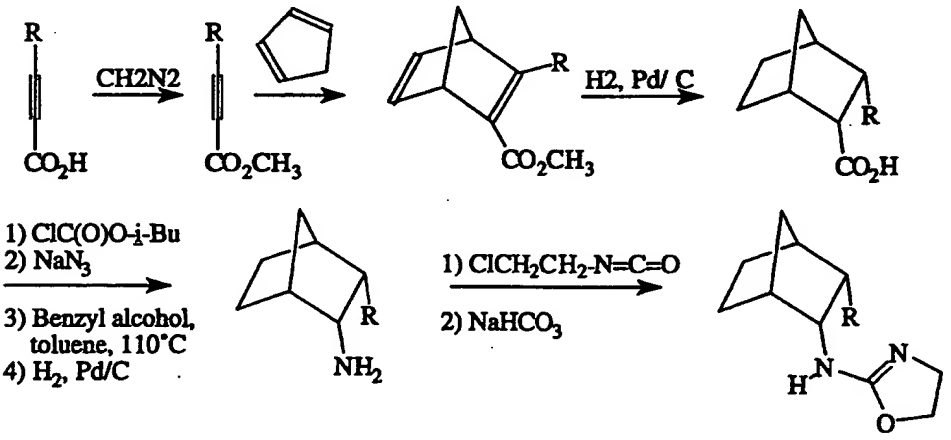
A second preferred group of compounds of the invention are those that incorporate the bicyclo[2.2.1]heptane group in their structure as the ring A group.

Within either of the two preceding preferred groups, a still more preferred embodiment is of compounds which have a hydrogen atom or an aromatic group at the position represented by R.

Methods of Preparation

As illustrated by Scheme I below, treatment of an alkynyl acid with diazomethane in ether afforded the corresponding ester. The ester and cyclopentadiene were warmed at 175° C for 40 hours to form the cycloadduct. This adduct was unstable to SiO_2 chromatography and was best purified using a Kugelrohr distillation.

SCHEME I



The double bonds in the cycloadduct were immediately saturated by treatment with H_2 and Pd/C at one atmosphere. Conversion of the ester into an amine was accomplished by conversion to the carboxylic acid followed by a Curtius reaction. Thus, the acid was activated by treatment with isobutylchloroformate. The acyl azide was formed by treatment with sodium azide. Elimination of nitrogen and formation of a benzyl carbamate occurred when the azide was warmed in toluene in the presence of benzyl alcohol. The amine was liberated upon treatment with H_2 and Pd/C at one atmosphere. Oxazoline synthesis

was accomplished under standard conditions: treatment first with chloroethylisocyanate and then aqueous $NaHCO_3$ solution.

endo, exo Relative stereochemistry

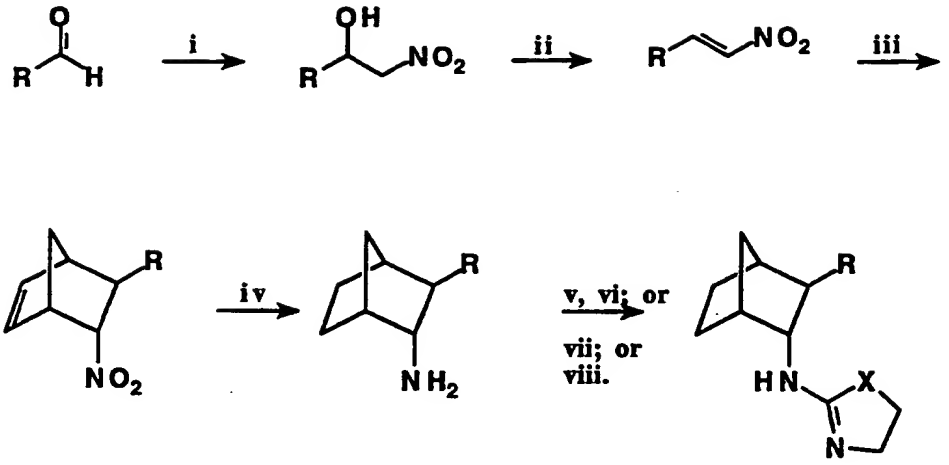
Preparation of β-nitrostyrene was accomplished according to the Organic Syntheses method. Treatment of a methanol solution of benzaldehyde with nitromethane (100 mol.-%) in the presence of sodium hydroxide (105 mol.-%) afforded the nitro alcohol. Dehydration of the alcohol was effected by treatment with aqueous hydrochloric acid (5M).

The nitrostyrene of 3,4-dihydroxybenzaldehyde was obtained by treating piperonal (3,4-methylenedioxybenzaldehyde) in a similar fashion to that reported for β-nitrostyrene. The acetal proved stable to the aqueous acid required for dehydration.

Construction of the bicyclo[2.2.1]heptane skeleton was carried out in two steps. The Diels-Alder reaction was conducted by warming the nitrostyrene with cyclopentadiene (110 mol.-%) neat (β-nitrostyrene is a low melting material) or in 1,2-dichloroethane (1M in nitroolefin). The Diels-Alder reaction proceeds in approximately a 3:1 *endo* nitro: *exo* nitro ratio. Both the ratio and relative stereochemistry was demonstrated through X-ray analysis. Reduction of both the nitro group and the olefin was carried under an atmosphere of hydrogen in the presence of 10 weight-% palladium on charcoal (10%). Separation of isomers was conveniently carried out at this stage using flash chromatography.

Oxazoline synthesis was conducted under standard conditions. The amine was first converted to the chloroethylurea by treatment with chloroethylisocyanate. Warming the chloroethylurea in the presence of sodium bicarbonate afforded the oxazolines. This effort is summarized in Scheme II. Thiazolines and imidazolines were also prepared under standard conditions. Treatment of amines with chloroethylisothiocyanate affords thiazolines directly while treatment with imidazoline-2-sulfonic acid affords the corresponding imidazolines in a single step.

Scheme II



Reagents and Conditions: i. CH3NO2, KOH, MeOH; ii. HCl; iii. cyclopentadiene, neat or 1M in dichloroethane; iv. H2, 10 Pd on C; v. chloroethylisocyanate; vi. NaHCO3 [X = O]; vii. chloroethylisothiocyanate [X = S]; viii. imidazoline-2-sulfonic acid [X = NH].

Synthesis of oxabicyclo[2.2.1]heptane derivatives of the present invention can also be prepared by Diels Alder reactions following means well known in the art. Grieco, Zelle, Lis and Finn in Journal of the American Chemical Society, 105, 1403-4 (1985) report means of making suitably derivatized oxabicyclo[2.2.1]heptane and oxabicyclo[2.2.1]heptene compounds which can be elaborated into compounds of the present invention. This can be accomplished by the synthetic steps which follow the Diels Alder cycloaddition in Scheme 1 using the 2-carbomethoxy-bicyclo[2.2.1]hept-2-ene intermediate of the reference, or if the nitro functionality of other of the Grieco et al. compounds are employed , according to the steps iv, v, vi (or vii or viii) in Scheme 2. Another journal article by Jarvest and Readshaw disclose advantageous conditions for Diels-Alder cyclization of derivatized furans and cyanoacrylate to yield 2-cyano-5-substituted-bicyclo[2.2.1]heptanes. These articles are incorporated by reference herein in their entirety.

The invention is further illustrated by the following non-limiting examples which are illustrative of a specific mode of practicing the invention and are not intended as limiting the scope of the appended claims.

Example 1.

2-Hydroxy-1-nitrohexane

Pentanal (49.6 ml, 464 mmol) was stirred in a solution of nitromethane (276 ml, 5108 mmol). To the reaction methanolic KOH (3N) was added dropwise to pH 8. The reaction was stirred at room temperature overnight. The solution contained trace amounts of insoluble dark brown material. The solution was washed with H_20 and extracted into dichloromethane; concentration of the solvent gave clean product (II) in 96% yield, (56.58g).

Example 2.

1-Nitrohex-1-ene

The nitroalcohol (1) (2.0 g, 13.6 mmol) was dissolved in dichloromethane and treated at 0°C for 30 minutes by dropwise addition with methanesulfonyl chloride (1.6 g, 13.6 mmol). Triethylamine (2.75 g, 27.2 mmol) was then added dropwise and stirred for an additional hour at 0°C. The product was washed with 1M H_3PO_4 and then with saturated $NaHCO_3$ and extracted with dichloromethane. Concentration of the solvent gave the olefin in 80% yield (3.03g).

Example 3.

Trans-2-nitro-3-butyl bicyclo[2.2.1]heptane

The nitroolefin (2) (3.00 g, 19.3 mmol) was dissolved in 20 mL of dichloromethane and then freshly cracked cyclopentadiene (6.49, 96.6 mmol) was added and bubbled with argon for 15 minutes. This was added to a sealable tube and once sealed was placed in an oil bath at 90°C overnight. The reaction went to completion. Excess cyclopentadiene was removed by Kugelrohr distillation. The resultant product was obtained in 60% yield (3.53g).

Example 4.

Trans 2-(3-butyl-bicyclo[2.2.1]heptyl)amine

The cycloadduct (3) (2.53g, 13.0 mmol) was dissolved in methanol (25 ml) and bubbled with Ar. To this was added 10% palladium on carbon (500 mg). This vessel containing this mixture was put on a Parr apparatus for hydrogenation at 50 psi overnight. The reduced material was filtered through celite and the solvent was concentrated. The residue was dissolved in 1M H_3PO_4 and washed with dichloromethane. The aqueous layer was basified with 25% NaOH to a pH of ca. 13. This was extracted with dichloromethane three times. The organic layers were combined and concentrated to give the product in 86% yield (1.86g).

Example 5A.

Trans 2-(3-butyl bicyclo[2.2.1]heptyl)amino-oxazoline

The amine (4) (200 mg, 1.20 mmol) was dissolved in THF (5 ml). To this was added chloroethylisocyanate (0.122 ml, 1.40 mmol) dropwise and stirred at room temperature for two hours. The reaction mixture was poured into 1M H_3PO_4 and ice (1:1) to quench the reaction. This was then extracted with dichloromethane and concentrated to give the urea. The urea was treated with methanol (6 ml), water (6 ml) and $NaHCO_3$ (202 mg, 2.4 mmol). This mixture was refluxed at 80°C for 2 hours. The reaction was quenched with saturated $NaHCO_3$ and extracted with dichloromethane. The organic layers were combined and concentrated to give desired product (270 mg). Column chromatography with 5% MeOH saturated with NH_3 in dichloromethane gave the desired product in 60% yield (155 mg).
^1H NMR(CDCl3): 0.70-1.70(M,16H), 1.9(d, 1H), 2.5(5, 1H), 3.4(S1H) 3.75(t,2H), 4.1(5,1H), 4.25(t,2H).

Elemental analysis: theoretical - C 71.14% H 10.23% N 11.86%
found - C 70.8% H 10.20% N 11.60%

5B.

Trans 2-(3-butyl-bicyclo[2.2.1]heptyl)aminothiazoline

The amine (4) (200 mg, 1.20 mmol) in THF (5 ml) was treated with chloroethylisothiocyanate dropwise at 0°C for 3 hours. The reaction mixture was poured into 1M H_3PO_4. The aqueous layer was extracted with dichloromethane and then basified with 25% NaOH to pH 13. The

aqueous layer was then extracted with dichloromethane three times. The organic layers were combined and concentrated to give the product in 11.6% yield (35 mg).

^{1}H NMR (CDCl3): 0.85 (t, 3H), 1.1-1.7(M, 13H), 1.95 (d,1H), 2.5 (S, 1H) 3.3 (t,2H), 3.5 (S,1H), 4.0 (t, 2H)

^{13}C NMR C (CD_3OD): δ 14.0, 21.0, 23.0, 30.0, 30.5, 35.0, 35.2, 35.3, 40.5, 41.5, 51.5, 64.0.

Elemental analysis:	theoretical	- C 66.63% H 9.59% N 11.10%
	found	- C 66.40% H 9.52% N 11.0%

5C.

Trans 2-(3-butyl-bicyclo[2.2.1]heptyl)aminoimidazoline

An acetonitrile (2.4 ml) suspension of the amine (4) (200 mg, 1.20 mmol) with triethylamine (0.184 ml, 1.32 mmol) and then with imidazoline-2-sulfonic acid (198 mg, 1.32 mmol). The solution was refluxed for 2 hours. Aqueous workup with 1 M H_3PO_4 and then basifying aqueous layer to pH 13 and extraction with dichloromethane gave the desired product. The HCl salt was prepared from HCl/ether in methanol which gave a yield of 20% (60 mg).

^{1}H NMR (CDCl3): 0.70-1.70 (M, 16H), 2.0 (d, 1H), 2.6 (S,1H), 3.4(S,1H) 3.65(S,4H)

^{13}C NMR ($CHCl_3$): δ 22.9, 27.8, 27.89, 28.05, 31.83, 32.29, 33.41, 37.34, 39.89, 42.53, 42.89, 44.06, 44.54, 57.9, 61.6, 95.6, 161.02.

Example 6.

2-Carbomethoxy-3-ethyl [2.2.1] bicyclo $\Delta^{2,3}$, $\Delta^{5,6}$ heptadiene

Methyl pent-2-yn-oate (5.3 g, 126.16 mmol) was dissolved in toluene (30 ml) and placed in a sealable tube. To this was added freshly cracked cyclopentadiene. The tube was sealed and placed in a oil bath at 168°C for 42 hours. The excess cyclopentadiene was removed by Kugelrohr distillation. The product was isolated in 70.3% yield (5.26 g).

Example 7.

Cis 2-carbomethoxy-3-ethyl[2.2.1]bicycloheptane

The cycloadduct (6) (5.26 g, 29.5 mmol) was dissolved in MeOH (60 ml) and bubbled with Ar, and to the solution was added 10% palladium on carbon (500 mg). The reaction vessel containing this mixture was put on a Parr apparatus for hydrogenation at 50 psi overnight. The reduced material was filtered through celite and solvent concentrated. The residue was dissolved in 1M H_3PO_4 and washed with dichloromethane. The aqueous layer was basified with 25% NaOH to ca. pH 13. This was extracted with dichloromethane three times. The organic layers were combined and concentrated to give product in 81% yield (4.7 g).

Example 8.

Cis 2(3-ethyl-bicyclo[2.2.1]heptyl) amine

The ester (3) (2.0 g, 10.2 mmol) was dissolved in a MeOH/THF (30 ml/20 ml) solution. This was treated with 2N LiOH (10.2 ml, 20.4 mmol) in H_2O at 100°C and refluxed. The reaction was concentrated to a paste and dissolved in 40 ml H_2O and washed twice with dichloromethane. The organic layers were combined and concentrated to give the corresponding acid. This acid was dissolved in acetone (20 ml), and triethylamine (3.06 ml, 22.1 mmol) was added dropwise. Next ethylchloroformate was added dropwise (2 ml, 20.9 mmol) at 0°C. The reaction was stirred for 1 hour. NaN_3 (676 mg, 10.4 mmol) was added in portions at 0°C for an additional hour. The reaction was partitioned between ice water and dichloromethane. The organic layers were combined and concentrated to give the acyl azide. This was then treated with benzyl alcohol (995 mg, 9.2 mmol) in toluene and refluxed at 110°C for 30 minutes. The reaction was washed with H_2O and extracted in dichloromethane. Concentration of solvent gave the benzyl carbamate. The carbamate was reduced in the same manner as before with 10% palladium on carbon. The product was obtained in an overall yield of 45% (550 mg).

NMR $H^1(CDCl_3)$: 0..8(t, 3H), 1.0-1.6(m, 9H), 1.8 (s,1H), 2.2 (s,1H), 4.7(s,1H), 5.3(s,2H)

Example 9A

Cis 2-(3-ethyl-bicyclo[2.2.1]heptyl) amino-oxazoline

The bicyclic amine (8) was treated as in the procedure outlined for the preparation of the trans compound (5A) above.
^{1}H-NMR ($CDCl_3$): 0.8(t, 3H), 1.00-2.00(m, 9H), 2.1(s,1H), 2.5(s,1H), 3.9(s,1H), 3.8(t, 2H), 4.2(t, 2H).

^{13}C NMR ($CDCl_3$): δ 14.5, 20.8, 25.5, 28.2, 38.2, 40.3, 44.7, 54.42, 55.05, 64.3, 69.2.
Analysis calculated for $C_{12}H_{20}N_2O$: C 69.09, H 9.68, N 13.45
Found: C 68.6, H 9.24, N 13.45.

9B.

Cis 2-(3-ethyl bicyclo[2.2.1]heptyl) aminothiazoline

can be prepared by substituting the bicyclic amine (8) for (4) in the preparation of 5B above.

9C.

Cis 2-(3-ethyl-bicyclo[2.2.1]heptyl) aminoimidazoline

Likewise, 9C can be prepared by substituting the bicyclic amine (8) for (4) in the preparation of 5C above.

Example 10

2-N-Bornyhlamino-oxazoline

To a THF solution of the amine (250 mg, 1.63 mmol) at 0° C was added chloroethylisocyanate (189 mg, 1.79 mmol) dropwise. The reaction was allowd to warm to r.t. and after stirring for one hr., all starting material was consumed. The reaction mixture was poured into 1M H3PO4 and extracted three times with methylene chloride. After drying, the solution was concentrated and the resulting solid was warmed in aqueous methanolic NaHCO3. The reaction was extracted from 0.5N NaOH and dried (Na2SO4), c ncentrated and chromatographed over 250-400 mesh silica using 5% ammonia saturated m thanol in CH_2Cl_2 as eluent. Yield: 206 mg (60%).

^{13}C NMR ($CDCl_3$) 161.7, 67.6, 57.8, 52.9, 49.2, 48.0, 44.8, 38.4, 28.3, 27.6, 19.9, 18.7, 13.7

^{1}H NMR ($CDCl_3$) 4.24 (2H, m); 3.80 (3H, m); 2.38 (1H, m); 1.87-1.1 (6H, env, m); 0.93 (3H, s); 0.87 (3H, s); 0.86 (3H, s)

Example 11.

Bicyclo[2.2.2]octane aminooxazoline

Adamantylaminooxazoline

In a similar manner to Example 10, commercially available bicyclo[2.2.2]octane amine and adamantylamine can be used to prepare the 2-bicyclo[2.2.2]octane-aminooxazoline and adamantylamino-oxazoline compounds, respectively.

Example 12.

Receptor Binding Assays

A.

Tissue preparation: Membrane suspensions were prepared from human cerebral cortex (HCC) obtained from the UCI Organ and Tissue Bank and rat kidney cortex (RKC). Briefly, tissues (1g) were homogenized in 25 mls of iced-cold 5 mM tris, pH 7.4 with a Polytron homogenizer for 30 secs at setting # 7, and centrifuged for 10-12 minutes at 300 x g at 4°C. The supernatant was filtered through 2 layers of gauze and diluted 1: 2 with 50 mM Tris-HCl buffer, pH 7.4, then centrifuged at 49,000 x g for 20 minutes. The pellet fraction was washed 3 times (resuspended in Tris-HCl buffer and centrifuged for 20 minutes at 49,000 x g). The pellet was then stored at -80°C until the binding assay.

Cell preparation: HT-29 and chinese hamster ovary (CHO) cells expressing the human α_{2A} (CHO-C10) receptor and CHO cells (CHO-RNG) expressing the rat α_{2B} adrenoceptor were grown to near confluency in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum using standard cell culture methods. Cells were harvested by scraping and placed into cold buffer of the following composition: 50 mM Tris-HCl, 5 mM EDTA, pH 7.4). Cells were then homogenized with a Polytron homogenizer for 2 X 10 secs at setting #

7, and centrifuged for 20 minutes at 49,000 x g. The pellet fraction was washed (resuspended in Tris-HCl, pH 8 buffer and centrifuged for 15-20 minutes at 49,000 x g) 2 times and stored at -100°C until binding assay.

Binding studies: The radioligands [^{3}H]rauwolscine (specific activity 80 Ci/mmol) and [^{3}H]prazosin (specific activity 76 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Frozen membrane pellet was resuspended in 25 mM glycine / glycine, pH 7.4 and incubated with radioligand under the following conditions: CHO-C10, CHO-RNG, HT-29 - [^{3}H]rauwolscine, 22 °C, 30 minutes; RKC-[^{3}H]rauwolscine, 0 °C, 120 minutes; and, HCC-[^{3}H]prazosin, 22 °C, 30 minutes in a final volume of 500 µL. At the end of the incubation period, the samples were filtered through glass fiber filters (Whatman GF/B) in a 96 well cell harvester and rapidly washed four times with 4 mL of iced-cold 50 mM Tris-HCl buffer. The filters were then oven dried and transferred to scintillation vials containing 5 mL of Beckman's Ready Protein® scintillation cocktail for counting. Specific binding defined by 10 µM phentolamine for competition studies were as follows: 0.3 nM [^{3}H]rauwolscine - CHO-C10 99%; 0.4 nM [^{3}H]rauwolscine - CHO-RNG 99%; 0.7 nM [^{3}H]rauwolscine - HT-29 90%; 1 nM [^{3}H]rauwolscine - RKC 92 %, and 0.3 nM [^{3}H]prazosin - HCC 87%. Protein concentrations were determined with a protein assay kit from Bio Rad. Binding isotherms, equilibrium dissociation and affinity constants were analyzed and determined by the non-linear least squares curve fitting programs AccuFit Competition / Saturation by Beckman.

Binding studies: The radioligands [^{3}H]rauwolscine (specific activity 80 Ci/mmol), [^{3}H]prazosin (specific activity 76 Ci/mmol) and [^{3}H]brimonidine (UK-14,304; specific activity 63 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Frozen membrane pellet was resuspended in either 50 mM tris, 2 mM EGTA, 1 mM $MgCl_2$, pH 7.5 (RbKC, RbICB-[^{3}H]brimonidine); 50 mM tris, 0.5 mM EDTA, 5 mM NaCl, pH 7.7 (RbICB-[^{3}H]rauwolscine); 25 mM glycine/glycine, pH 7.4 (RtKC, CHO-C10, CHO-RNG, HT-29, HCC) or 50 mM tris, 0.1 mM $MnCl_2$, pH 7.7 (RtCC). Membrane protein homogenate (75 - 200 µg) was incubated with radioligand under the following conditions: RbKC and RbICB-[^{3}H]rauwolscine, 22 °C, 45 minutes; RtCC and RbICB-[^{3}H]brimondine, 22 °C, 90 minutes; CHO-

C10, CHO-RNG and HT-29-[^{3}H]rauwolscine, 22 °C, 30 minutes; HCC-[^{3}H]prazosin, 22 °C, 30 minutes; and , in a final volume of 250 or 500 µL. At the end of the incubation period, the samples were filtered through glass fiber filters (Whatman GF/B) in a 24 or 96 well cell harvester and rapidly washed four times with 4 mL of iced-cold 50 mM Tris-HCl buffer. The filters were then oven dried and transferred to scintillation vials containing 10 mL of Beckman's Ready Protein® scintillation cocktail for counting. Specific binding defined by 10 µM phentolamine for competition studies were as follows: 2.4 nM [^{3}H]brimonidine-RbICB 62 %; 2.4 nM [^{3}H]rauwolscine-RbICB 75 %; 2 nM [^{3}H]rauwolscine-RbKC 88 %; ; 0.3 nM [^{3}H]rauwolscine-CHO-C10 99%; 0.4 nM [^{3}H]rauwolscine-CHO-RNG 99%, , 0.3 nM [^{3}H]prazosin 87%; and 1 nM [^{3}H]rauwolscine-RtCC 90%. Protein concentrations were determined with a protein assay kit from Bio Rad. Binding isotherms, equilibrium dissociation and affinity constants were analyzed and determined by the non-linear least squares curve fitting programs EBDA (BioSoft) or AccuFit Competition / Saturation by Beckman.

B.

Cell preparation: Chinese hamster ovary (CHO) cells expressing the human α_{2A} (CHO-C10) and the rat α_{2B} (CHO-RNG) adrenoceptors were grown to near confluency in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum using standard cell culture methods. Cells were harvested by scraping and placed into cold buffer of the following composition: 50 mM Tris-HCl, 5 mM EDTA, pH 7.4). Cells were then homogenize with a Polytron homogenizer for 2 X 10 sec at setting #7, and centrifuged for 20 minutes at 49,000 x g. The pellet fraction was washed (resuspended in Tris-HCl, pH 8 buffer and centrifuged for 15-20 minutes at 49,000 x g) 2 times and stored at -100°C until binding assay.

C.

Binding studies: Determination of K_i

The radioligands [^{3}H]rauwolscine (specific activity 80 Ci/mmol) and [^{3}H]prazosin (specific activity 76 Ci/mmol) were obtained from New England Nuclear, Boston, MA. Frozen membrane pellet was resuspended in 25 mM glycine/glycine, pH 7.4 and incubated with radioligand under the following conditions: CHO-C10, CHO-RNG - [^{3}H]rauwolscine, 22 °C, 30 minutes; and, HCC-[^{3}H]prazosin, 22 °C, 30 minutes in a final volume of 500 µL. At the end of the incubation

period, the samples were filtered through glass fiber filters (Whatman GF/B) in a 96 well cell harvester and rapidly washed four times with 4 mL of iced-cold 50 mM Tris-HCl buffer. The filters were then oven dried and transferred to scintillation vials containing 5 mL of Beckman's Ready Protein® scintillation cocktail for counting. Specific binding defined by 10 μM phentolamine for competition studies were as follows: 0.3 nM [^{3}H]rauwolscine-CHO-C10 99%; 0.4 nM [^{3}H]rauwolscine-CHO-RNG 99%, and 0.3 nM [^{3}H]prazosin - HCC 87%. Protein concentrations were determined with a protein assay kit from Bio Rad. Binding isotherms, equilibrium dissociation and affinity constants were analyzed and determined by the non-linear least squares curve fitting programs AccuFit Competition/Saturation by Beckman.

Determination of α_2 activation: Measuring efficacy (EC_{50})

Vas Deferens: The prostatic ends of the vas deferens (2-3 cm) were removed from albino rabbits and mounted between platinum electrodes in 9 ml organ baths containing Krebs-Hensleit solution of the following composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2. $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. This solution was maintained at 35° C and bubbled with 95% O_2 and 5% CO_2. The tissue was equilibrated at 0.5 g tension for 30 minutes. The vas deferens strips were then field stimulated at 0.1 Hz, 2 msec, 90 mA using a square wave stimulator (World Precision Instruments A310 Accupulser / A385 Stimulus Isolater), or a Grass S48 stimulator at 0.1 Hz, 2 msec, 70 volts. After 30 minutes of electrical stimulation, cumulative concentration-response curves in 0.25 log units were obtained with a 4 minute contact time for each concentration. Each tissue was used to evaluate only one drug. Tissue contractions produced by the field stimulation were measured isometrically using Grass FT-.03 force-displacement transducers and recorded on a Grass Model 7D physiograph. The reduction in electrically-evoked peak height by the drugs was measured and expressed as a percentage of the pre-drug peak height. The IC_{50} was determined as the concentration which produced a 50% reduction in peak height.

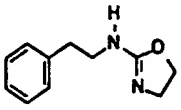
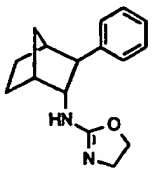
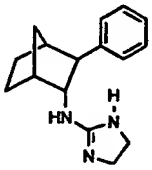
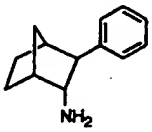
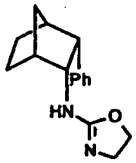
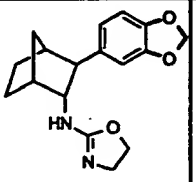
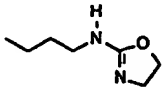
<u>I_1 imidazoline receptor binding assay</u>

Fresh bovine brain stems were obtained from a local slaughter house. After removal of pia-arachnoid, the medulla was isolated by dissecting the brain stem about 1 cm posterior and 1 cm caudal to the obex. The ventral quadrants of the medulla excluding the pyramids were used as the VLM. For each preparation, 30 to 40 VLM were used. Initial homogenization was performed in 20 volumes of 5 mM HEPES buffer (pH 7.4 at 4°C) containing 250 mM sucrose, 50 uM Calpain I inhibitor (Boehginer Mannheim, Indianapolis, IN), 100 uM 1,10-phenanthroline (Sigma, St. Louis, MO) and 50 uM Pefabloc (Boehginer Mannheim, Indianapolis, IN), using Virtis homogenizer at setting 7 with three 10 second pulses, followed by three passes in a teflon-glass tissue homogenizer. The inhibitors were added to prevent degradation by proteases and peptidases. The homogenates were then centrifuged at 1000xg for 10 min and the resulting pellets were re-homogenized and centrifuged. Supernatants resulted from both runs were combined and centrifuged for 20 min at 48000xg. The pellets obtained were resuspended in a teflon-glass homogenizer in 50 mM Tris-HCl with 5 mM EDTA (pH 7.7 at 4°C), centrifuged, and resuspended in 50 mM Tris-HCl with 25 mM NaCl, pH 7.7 at room temp. To remove endogenous ligands, the homogenate was incubated 30 min at room temp before it was centrifuged for 20 min at 48000xg. The pellets were then washed with 50 mM Tris-HCl buffer, (pH 7.4 at 4°C) and loaded on top of 5 mM HEPES/0.85 M sucrose (pH 7.4 at 4°C). Pellets obtained after centrifugation at 48000xg for 20 min were saved. The fatty connective tissue on the top layer was discarded. The partially purified VLM membrane pellets were then washed twice with 50 mM Tris-HCl, pH 7.7 at 4°C, flash frozen in dry ice/acetone slush, and stored at -100°C until use. Receptor binding experiments were performed within days after the membrane preparation.

I_1 imidazoline receptor binding affinity was determined from radioligand binding of ^{3}H-clonidine (NEN, Boston, MA) to bovine VLM membranes. Specific activity of ^{3}H-clonidine was 43 Ci/mmol. Kd of ^{3}H-clonidine binding to the I_1 imidazoline receptor was determined by saturation experiments and Ki of other ligands studied were determined by competition experiments. The radioligand binding assay was performed in Teflon 96-wells with the Biomek-1000 robotics (Beckman Instruments, Fullerton, CA). Each well contained 4 nM ^{3}H-

Clonidine and 0.3 to 0.5 mg of bovine VLM protein in 5 mM HEPES buffer containing 0.5 mM EGTA and 0.5 mM $MgCl_2$, pH 7.4 (0.1 mM ascorbic acid was added just before the protein). After 50 min of incubation at 25°C, the reaction was terminated by rapid filtration over Whatman GF/B filters treated with 0.1% polyethyleneimine and washed with 12 ml ice cold 50 mM Tris-HCl, pH 7.4 at 4°C in a Brandel Harvester (Brandel, Gaithersburg, MD). Both 'hot' and 'cold' saturation experiments were performed. In 'hot' saturation experiments, studies were performed with ^{3}H-clonidine ranging from 0.1 to 50 nM. In 'cold' saturation experiments, studies were performed with 2 nM ^{3}H-clonidine with 20 different concentrations of the unlabeled clonidine, ranging from 0.1 nM to 1 uM unlabeled clonidine. Non specific binding was defined by parallel incubations containing 10^{-5} M phentolamine or naphazoline. Imidazoline binding was determined by parallel incubations in which the alpha-adrenergic sites were masked with 10^{-5} M norepinephrine. During competition experiments, ligands of 20 concentrations ranged from 10^{-11} to 10^{-4} were used. Radioactivity was counted in a Beckman LS-3801 scintillation counter. Data were captured and analyzed with Accufit saturation and competition software modeled both for one-site and two-site fits (Beckman Instruments, Fullerton, CA) using an IBM compatible computer. All experiments were repeated at least twice.

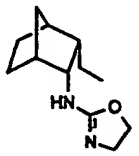
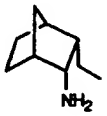
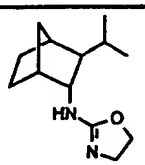
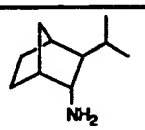
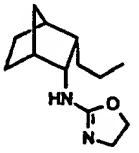
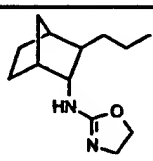
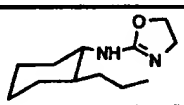
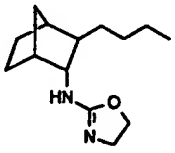
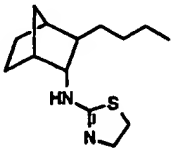
TABLE I

STRUCTURE TESTED	K_i (nM)				EC_{50} (nM)
	α_1	α_{2A} (CHO-C10) (HT-29)*	α_{2B} (CHO-RNG) (RKC)†	I_1	α_2 (VAS DEFERENS)
	11,131	1,751	4,174	—	>56,200
	1,864	3.1	7.8	12	29,000
	6,730	14.3	72	100,000	>56,200
	10,977	6,571	6,103	350	not tested –no α_2 binding observed
	73	1.9	27	2.5	3,700
	1,860	1.1	4.6	—	>56,000
	>100,000	150*	317†	—	1,100

5

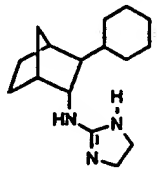
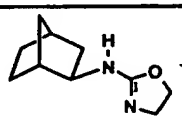
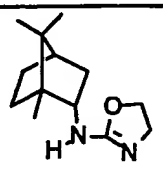
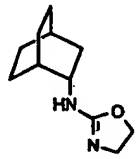
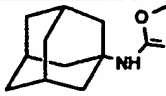
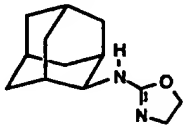
* HT-29(α_{2A})

† Rat Kidney Cortex (α_{2B})

STRUCTURE TESTED	K_i (nM)				EC_{50} (nM)
	α_1	α_{2A} (CHO-C10) (HT-29)*	α_{2B} (CHO-RNG) (RKC)†	I_1	α_2 (VAS DEFERENS)
	24,760	59	616	404	not tested
	>100,000	67,247	57,075	556	not tested –no α_2 binding observed
	>10,000	43*	58†	—	not tested
	>100,000	23,320	20,950	42	not tested
	8,600	25	256	3.8	903
	8,851	9.8	28.1	—	46,000
	1,600	0.6	8.3	—	1.0
	5,824	29*	59†	—	not tested
	42,000	58*	167†	—	not tested

* HT-29(α_{2A})

† Rat Kidney Cortex (α_{2B})

STRUCTURE TESTED	K_i (nM)				EC_{50} (nM)
	α_1	α_{2A} (CHO-C10) (HT-29)*	α_{2B} (CHO-RNG) (RKC)†	I_1	α_2 (VAS DEFERENS)
	17,240	288*	5,100†	—	not tested
	>10,000	4.2	11.5	328	>10,000
	>10,000	368	1,935	—	16,000
	32,487	119	770	—	>10,000
	>10,000	102	358	69,720	>5,000
	34,950	352	1,838	—	>10,000

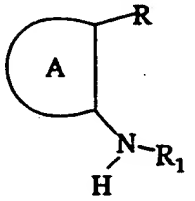
Several modifications of the above described compounds, the processes disclosed for making them, and application of the disclosed processes to numerous compounds beyond the examples set forth above, may be practiced by those skilled in the art without departing from the scope and spirit of the present invention. Therefore the scope of the present invention should be interpreted solely from the following claims, as such claims are read in light of the present disclosure.

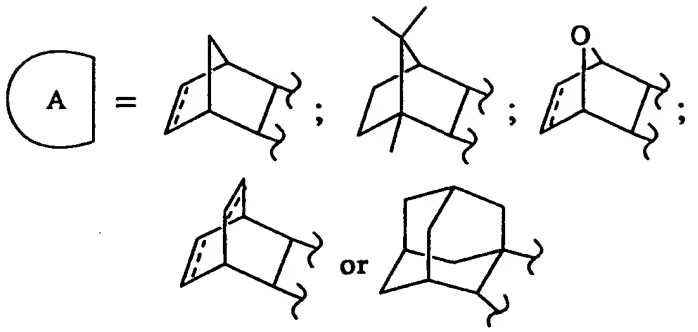
* HT-29(α_{2A})

† Rat Kidney Cortex (α_{2AB})

What is claimed is:

1. A compound of formula I





I

in which: ring A is one of the five alternative multi-cyclic rings as shown wherein a dotted line adjacent to a bond indicates that a single bond or a double bond may be present at that position; R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline; R is hydrogen, straight or branched chain alkyl of 1 to 6 carbon atoms, or straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl; or a stereoisomer, or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein R_1 is 2-oxazoline.

3. The compound of claim 1 wherein R_1 is 2-imidazoline.

4. The compound of claim 1 wherein R_1 is 2-thiazoline.

5. The compound of claim 1 wherein the ring A is bicyclo[2.2.1]heptane (norbornane).

6. The compound of claim 2 wherein the ring A is bicyclo[2.2.1]heptane (norbornane).

7. The compound of claim 5 wherein R is hydrogen; phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms; or methylenedioxyphenyl.

8. The compound of claim 1 which is trans-2-(3-phenyl-bicyclo[2.2.1]heptyl)-2-aminooxazoline.

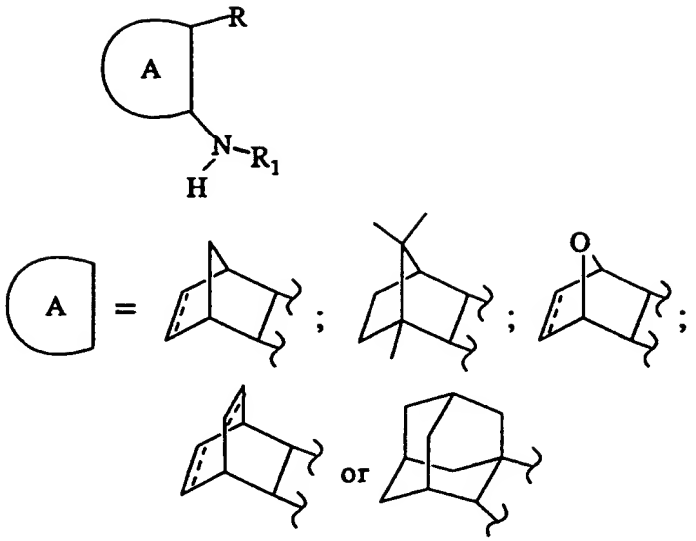
9. The compound of claim 1 which is trans-2-(3-phenyl-bicyclo[2.2.1]heptyl)-2-aminoimidazoline.

10. The compound of claim 1 which is trans- 2-(3-methylenedioxy-phenyl-bicyclo[2.2.1]heptyl)-2-aminooxazoline.

11. The compound of claim 1 which is trans-2-(3-propyl-bicyclo[2.2.1]heptyl)-2-aminooxazoline.

12. The compound of claim 1 which is 2-cyclo[2.2.1]heptyl-2-aminooxazoline.

13. A composition suitable for administration to a mammal having a disease state which is alleviated by treatment with an α_2-blocking agent, the composition comprising a therapeutically effective amount of a compound of formula I

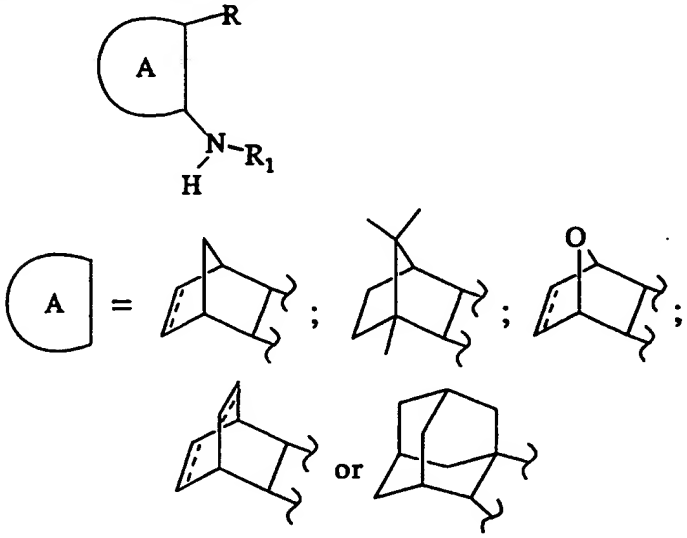


I

in which: ring A is one of the five alternative multi-cyclic rings as shown wherein a dotted line adjacent to a bond indicates that a single bond or a double bond may be present at that position; R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline; R is hydrogen, straight or branched chain alkyl of 1 to 6 carbon atoms, or straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl; or a stereoisomer, or a pharmaceutically acceptable salt thereof, in admixture with one or more pharmaceutically acceptable carriers.

14. The composition of claim 13 wherein the therapeutically effective compound is selected from the group consisting of trans-2-(3-phenyl-bicyclo[2.2.1]heptyl)-2-aminooxazoline, trans-2-(3-phenyl-bicyclo[2.2.1]heptyl)-2-aminoimidazoline, cis- 2-(3-phenyl-bicyclo[2.2.1] heptyl)-2-aminooxazoline, trans- 2-(3-methylenedioxy-phenyl-bicyclo [2.2.1]heptyl)-2-aminooxazoline, trans-2-(3-propyl-bicyclo[2.2.1]heptyl)-2-aminooxazoline, cis-2-(3-propyl-bicyclo[2.2.1] heptyl)-2-aminooxazoline and 2-cyclo[2.2.1]heptyl-2-aminooxazoline.

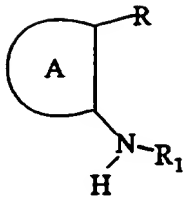
15. A method for treating a mammal having a disease state which is alleviated by treatment with an α_2 blocking agent, which comprises administering a therapeutically ffectiv amount of a compound of the formula I

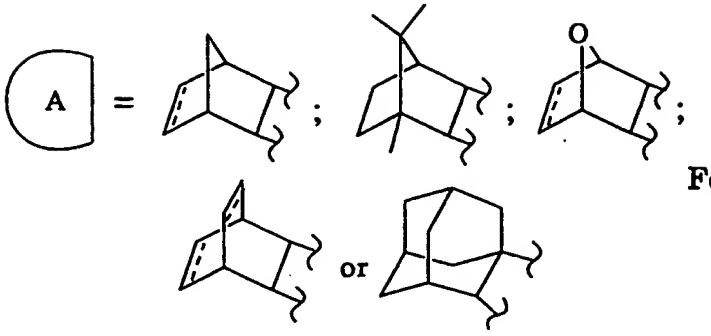


formula I

in which: ring A is one of the five alternative multi-cyclic rings as shown wherein a dotted line adjacent to a bond indicates that a single bond or a double bond may be present at that position; R_1 is 2-oxazoline, 2-imidazoline or 2-thiazoline; R is hydrogen, straight or branched chain alkyl of 1 to 6 carbon atoms, or straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl; or a stereoisomer or a pharmaceutically acceptable salt thereof, and wherein the disease state is chosen from the group consisting of mental depression, elevated intraocular pressure, non insulin-dependent diabetes, male impotence and obesity.

16. A method of protecting the ocular or brain nerve cells in a mammal suffering a noxious action or at risk of experiencing a noxious action on said nerve cells comprising administering to said mammal an effective amount of a compound of formula I to inhibit or prevent nerve cell injury or death





Formula I

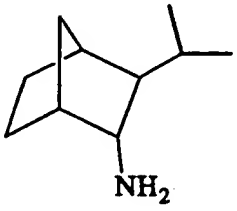
wherein ring A is one of the five alternative multi-cyclic rings as shown wherein a dotted line adjacent to a bond indicates that a single bond or a double bond may be present at that position; R_1 is hydrogen, 2-oxazoline, 2-imidazoline or 2-thiazoline; R is hydrogen, straight or branched chain alkyl of 1 to 6 carbon atoms, or straight or branched chain alkenyl of 2 to 6 carbon atoms, a cycloaliphatic ring of 3 to 6 carbon atoms, phenyl optionally mono- or di-substituted with hydroxy, halogen, alkyl of 1 to 3 carbon atoms or alkoxy of 1 to 2 carbon atoms, or methylenedioxyphenyl.

17. The method of claim 16 wherein R_1 is hydrogen.

18. The method of claim 16 wherein ring A is norbornane.

19. The method of claim 16 wherein R_1 is oxazoline.

20. The method of claim 16 wherein the compound of formula I is



INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 95/08733

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D263/28 C07D277/18 C07D233/50 A61K31/42 A61K31/13
A61K31/415 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,4 590 202 (DAVID C. REMY) 20 May 1986 see the whole document ---	1,13,15
A	EP,A,0 251 453 (BEECHAM GROUP PLC) 7 January 1988 cited in the application see page 11 - page 16; claims 1,14 ---	1,13,15
A	US,A,4 064 348 (NORTON P. PEET ET AL) 20 December 1977 see the whole document ---	1,13,15
A	US,A,3 598 833 (RUDOLF HILTMANN ET AL) 10 August 1971 cited in the application see the whole document --- -/--	1,13,15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 1995	27. 09. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Henry, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. .onal Application No
PCT/US 95/08733

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A,5 066 664 (CHARLES GLUCHOWSKI) 19 November 1991 cited in the application see the whole document -----	1,13-16

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US95/08733

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 15,16,18 and 19 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compounds as claimed in claims 1-14.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. .onal Application No
PCT/US 95/08733

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4590202	20-05-86	NONE		
EP-A-251453	07-01-88	AU-B-	600939	30-08-90
		AU-A-	7247987	05-11-87
		JP-A-	62267270	19-11-87
		US-A-	4861789	29-08-89
US-A-4064348	20-12-77	NONE		
US-A-3598833	10-08-71	BE-A-	704394	27-03-68
		CH-A-	487916	31-03-70
		CH-A-	487917	31-03-70
		DE-A-	1670753	16-07-70
		FR-M-	7818	20-05-70
		FR-A-	1543533	
		GB-A-	1147720	
		NL-A-	6713158	28-03-68
US-A-5066664	19-11-91	NONE		